Supplemental Figure legends

Supplemental Figure 1. BTK shRNAs are highly toxic for ABC DLBCL cells with wild type CARD11. Shown are the results of the shRNA barcode screen of the ABC DLBCL cell line OCI-Ly10 that has wild type CARD11. Shown are log2 fold differences in shRNA barcode abundance in induced vs. uninduced cultures. From a library of 3539 shRNAs, 135 were toxic for the OCI-Ly10 cell lines ($p<0.01$, log2 fold difference >0.8). Two separate BTK shRNAs were ranked #2 and #3 in this list, with the top ranking shRNA targeting the kinase WNK1.

Supplemental Figure 2. Knockdown of CD79A and CD79B kills ABC DLBCL cells. A. The degree of CD79A knockdown is correlated with the level of surface BCR in HBL-1 and BJAB cell lines transduced with 12 different CD79A shRNAs. Each symbol indicates a distinct shRNA sequence targeting CD79A, expressed in either the ABC DLBCL line HBL-1 or the GCB DLBCL line BJAB, as indicated. **B.** The degree of CD79B knockdown is correlated with the level of surface BCR in five clones of HBL-1 cells transduced with two CD79B shRNAs. In a survey of CD79B shRNAs, none blocked surface BCR expression as effectively as the CD79A shRNAs (data not shown). Therefore, HBL-1 cells were transduced with two different CD79B shRNAs and single cell clones were selected. **C.** Toxicity of CD79B knockdown in HBL-1 clones is proportional to the degree of CD79B knockdown and decrease in surface IgM (panel B). HBL-1 clones expressing CD79B shRNAs and GFP were admixed with an equal number of untransfected HBL-1 cells. shRNA expression was induced with doxycycline and the percentage of GFP+ cells relative to day 0 was measured over time, as indicated.

Supplemental Figure 3. Knockdown of IgM and Igκ by shRNAs. BJAB cells were transduced with indicated shRNAs. Surface expression of IgM or Igκ in transduced cells was evaluated by flow-cytometry and compared with that of untransduced cells.

Supplemental Figure 4. Knockdown of BCR signaling components inhibits expression of NF-kB signature genes. A. Gene expression profiling following

incubation of the ABC DLBCL cell line HBL-1 with the IKKβ inhibitor MLN120B[27] to define an NF-kB target gene signature. Gene expression profiling measurements of the indicated NF-kB target genes are depicted at various time points following ML120B treatment according to the color scale shown (left). These NF-kB target genes were measured by gene expression profiling in HBL-1 cells transduced with multiple shRNAs directed against BTK, CARD11, SYK, and CD79A, after shRNA induction for the indicated times (right). **B.** Decrease in the NF-kB target gene signature following knockdown of BCR signaling components. Gene expression measurements for the component genes in the NF-kB signature (panel A) were averaged for each of the indicated conditions. **C.** Chronic active BCR signaling induces IKK kinase activity. An IκBα-luciferase fusion protein was expressed in ABC DLBCL cell lines with chronic active BCR signaling (TMD8) or without (OCI-Ly3). Luciferase activity was measured in these IKK reporter lines at various time points following induction of the indicated shRNAs and compared with values from uninduced cells. A rise in the luciferase reporter indicates IKK inhibition.

Supplemental Figure 5. Knockdown of BCR pathway genes is toxic for ABC DLBCL cells with chronic active BCR signaling. A focused shRNA library targeting the indicated BCR pathway genes (12-15 shRNAs/gene) was screened in quadruplicate in two ABC DLBCL cell lines with chronic active BCR signaling (TMD8; HBL1) and two GCB DLBCL cell lines (OCI-Ly19; OCI-Ly7). Shown is relative shRNA abundance in the shRNA-uninduced population relative to the induced population. Representative shRNAs targeting BCR pathway genes that were significantly toxic for the ABC DLBCL cell lines are shown. Also shown is a negative control shRNA and positive control shRNAs targeting the ribosome (RPL6, RPS13), proteasome (PSMC3) or MYC that were toxic to all DLBCL cell lines. Bar values are mean +/- s.d. of four independent transductions.

Supplemental Figure 6. SYK kinase activity in DLBCL cell lines. A. SYK knockdown in various DLBCL cell lines. Each line was transduced with a retroviral vector expressing an shRNA targeting SYK or a control (ctrl) shRNA. Western blot

analysis of SYK protein expression was performed after doxycycline induction of shRNA expression for 2 days. **B.** SYK kinase activity is toxic for the ABC DLBCL cell line OCI-Ly10. Wild type or kinase-dead (K402R) SYK was expressed as a GFP fusion protein in the ABC DLBCL cell lines OCI-Ly10 and HBL-1 and in the GCB DLBCL cell line BJAB. Viable GFP+ cells were enumerated at various times following retroviral transduction and normalized to the day 2 values. SYK was selectively toxic for OCI-Ly10 cells, and this requires SYK kinase activity. **C.** The kinase inhibitor R406 is toxic for ABC and GCB DLBCL cell lines irrespective of SYK-dependence. The indicated cell lines were treated with a range of concentrations of R406 for four days. Viable cells were assessed using an MTT assay and normalized to values obtained in untreated cells.

Supplemental Figure 7. BCR cluster formation in ABC DLBCL. A. BCR clusters in primary biopsy samples from patients with ABC DLBCL. TIRF microscopy was used to study DLBCL cells from 3 patients with DLBCL, which had been assigned to the ABC DLBCL subtype based on gene expression profiling. For ABC DLBCL sample #3, the intensity of surface IgM staining was low. The white arrows highlight the faint BCR clusters present in this sample. The cell shown has BCR cluster. The average clusters per cell +/– s.e.m. are depicted for at least 20 cells from each sample analyzed. **B.** Colocalization of BCR clusters and phospho-tyrosine in ABC DLBCL. TIRF microscopy was used to visualize IgM and phospho-tyrosine in association with the plasma membrane in various DLBCL cell lines. A Pearson correlation coefficient was calculated between IgM and phospho-tyrosine staining based on analysis of 17-33 cells for each line. Strong correlation was seen in the ABC DLBCL cell lines HBL-1, TMD8 and U2932 but not GCB DLBCL cell lines. The OCI-Ly10 ABC DLBCL cell line gave an intermediate correlation, perhaps due to the relatively weak intensity of the surface IgM staining in this cell line. Since the two GCB DLBCL cell lines did not form clusters in the membrane and their BCRs were mobile (Fig. 2G), we interpret the low correlation between IgM and phospho-tyrosine staining as background in this assay.

Supplemental Figure 8. CD79B mutations in ABC DLBCL are somatically acquired. Shown are representative chromatograms from an ABC DLBCL tumor biopsy

(top) and the corresponding germ line DNA, with the somatically acquired CD79B mutation indicated. The cases for which we confirmed the somatic nature of the CD79B mutations are listed.

Supplemental Figure 9. Wild type and mutant CD79 can prevent cell death in ABC DLBCL. A. Rescue of OCI-Ly10 ABC DLBCL cells, harboring the CD79A$^{\Delta 191\text{-}208}$ mutation, from toxicity of a CD79A shRNA by wild type and mutant forms of CD79A. OCI-Ly10 cells were transduced with vectors expressing wild type or mutant CD79A coding regions along with a CD8 surface marker and were subsequently transduced with a vector expressing an shRNA targeting the CD79A 3' untranslated region (UTR) along with GFP. The percentage of live CD8+, GFP+ cells relative to the day 2 value is depicted. Control cells without CD79A reconstitution died upon CD79A knockdown (black line), but wild type CD79A and CD79$^{\Delta 191\text{-}208}$ rescued the cells equivalently, as indicated. **B.** Rescue of HBL-1 ABC DLBCL cells, harboring the CD79B^{Y196F} mutation, from toxicity of a CD79B shRNA by wild type or mutant forms of CD79B. An HBL-1 clone was engineered to express shRNAs targeting the CD79B 3'UTR in a doxycycline-inducible fashion along with constitutive GFP expression. This clone was transduced with vectors expressing wild type or mutant CD79B coding regions along with a CD8 surface marker. These cells were mixed 1:1 with unmodified HBL-1 cells and the percentage of CD8+, GFP+ cells relative to the day 2 value is depicted. CD79B knockdown was toxic to HBL-1 cells (black line), but ectopic provision of wild type CD79B or CD79B^{Y196F} rescued the cells comparably, as indicated.

Supplemental Figure 10. Putative role of CD79 mutations and chronic active BCR signaling in the genesis of ABC DLBCL. The acquisition of CD79 mutations is envisioned to occur early in the progression to malignancy in a B cell clone stimulated by a self or foreign antigen (red). The mutations may enhance BCR signaling and clonal expansion by promoting surface BCR expression and preventing negative autoregulation by LYN kinase. Spontaneous BCR clusters form in a manner that is independent of the CD79 mutations and may or may not depend upon antigen engagement. Finally, a number of oncogenic hits must be further acquired to create a fully malignant ABC

DLBCL, including most commonly deletion of the INK4a/ARF locus, amplification of BCL-2 and SPIB loci, and trisomy 3.

Supplemental Figure 11. BCR cluster formation in ABC DLBCL is independent of CD79A or CD79B mutations. A. DLBCL cell lines were transduced with GFP-tagged wild type or mutant CD79B, and GFP clusters were enumerated by TIRF microscopy. Wild type and mutant CD79B formed clusters equivalently in ABC DLBCL cell lines but did not form clusters in GCB DLBCL cell lines. **B.** Expression of endogenous CD79B was knocked down in BJAB GCB DLBCL cells or HBL-1 ABC DLBCL cells, and cells were reconstituted with the wild type CD79B or various mutant isoforms. Clustering of IgM was measured by TIRF microscopy. Wild type and mutant CD79B formed BCR clusters equivalently in the ABC DLBCL cell line but did not form clusters in the GCB DLBCL cell line. **C.** Expression of endogenous CD79A was knocked down in OCI-Ly10 ABC DLBCL cells, and cells were reconstituted with the wild type CD79A or two mutant isoforms. Clustering of IgM was measured by TIRF microscopy. Cells reconstituted with wild type or mutant CD79A formed BCR clusters equivalently whereas cells without CD79A reconstitution had few if any BCR clusters. The average clusters per cell +/– s.e.m. are depicted for at least 20 cells from each line analyzed.

Supplemental Figure 12. Dasatinib inhibits IKK in ABC DBLCLs with chronic active BCR signaling. Effect of dasatinib or an IKKβ inhibitor (MLN120B) on IKK activity in ABC DLBCL lines as measured using an IκBα-luciferase reporter. Dasatinib blocked IKK in the TMD8 reporter line with chronic active BCR signaling and not the BCR-independent OCI-Ly3 reporter line. The IKKβ inhibitor blocked IKK in both reporter lines. Controls were equivalent concentrations of the vehicle DMSO. See legend to Supplemental Fig. 4C.

Supplemental Figure 13. Synergistic killing of ABC DLBCLs with chronic active BCR signaling by rapamycin and an IKKβ inhibitor. The indicated cell lines were treated with the two small molecular inhibitors at a range of concentrations. Synergistic

killing was seen in ABC DLBCLs with chronic active BCR signaling (HBL-1, TMD8), but not in the BCR-independent line OCI-Ly3 or in the GCB DLBCL line BJAB.

Supplemental Figure 14. Assignment of DLBCL cell lines to the ABC and GCB subtypes. Shown are gene expression profiling data for cell lines not previously categorized[4]. Genes were chosen that were differentially expressed between ABC and GCB DLBCL primary biopsy samples (fold > 2x or <0.5x, $p<0.001$). TMD8 cells are clearly derived from ABC DLBCL whereas SUDHL4 and SUDHL10 are GCB DLBCLs.

Supplemental Figure 15. Specific mRNA and protein knockdown by shRNAs. A. shRNA-mediated knockdown of mRNA expression. Data shown are from gene expression profiling of HBL-1 cells transduced with the indicated shRNAs. Doxycycline was used to induce shRNA expression for 24 or 48 hours and gene expression was compared in induced versus parallel uninduced cultures. Each shRNA knocked down expression of its respective mRNA and not others. **B.** shRNA-mediated knockdown of protein expression. shRNA expression was induced in HBL-1 cells for 48 hours prior to Western blot analysis. Each shRNA knocked down expression of its respective protein.